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Antibiotic Susceptibility in Neonatal Invasive Isolates of *Streptococcus agalactiae* in a 2-Year Nationwide Surveillance Study in Germany

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The antimicrobial susceptibility of 296 invasive neonatal group B streptococcus isolates from a nationwide 2-year surveillance study in Germany was investigated. All isolates were susceptible to beta-lactams, linezolid, quinupristin-dalfopristin, and vancomycin. Erythromycin and clindamycin resistance was found in 10.1 and 5.7%, respectively. The *ermB*, *ermTR*, or *mefA* gene was detected in all but one of the erythromycin-resistant isolates.

Group B streptococcus (GBS) is the leading cause of sepsis and meningitis in neonates, with a high rate of mortality and permanent disability (27, 29). Penicillin is still the drug of choice for treatment of GBS infection. In most susceptibility studies penicillins are highly active against GBS, and tolerance has only infrequently been reported (1). Likewise, high-level aminoglycoside resistance has rarely been documented (4). In contrast, the increasing prevalence of macrolide resistance raises concern about the empirical use of these antibiotics for the prevention of GBS infections (11, 15, 18, 22, 26). The aims of this study were to determine the patterns of susceptibility of invasive GBS isolates in Germany to different classes of antibiotics and to characterize the mechanisms of macrolide resistance. This is the first report on a nationwide surveillance of invasive neonatal GBS disease in Germany.

Active surveillance for invasive GBS infections in all neonates up to the age of 3 months was performed from April 2001 through March 2003. Monthly questionnaires were sent out by the Laboratory Sentinel Group at the Robert Koch Institute, Berlin, Germany, to all microbiological laboratories serving pediatric hospitals throughout Germany. Laboratories were required to report any culture-positive blood or cerebrospinal fluid GBS infection in this age group and to send isolates to the central study laboratory. The overall response rate was 95%. All isolates were tested for penicillin, ampicillin, cefotaxime, erythromycin, clindamycin, gentamicin, linezolid, quinupristin-dalfopristin, imipenem, meropenem, ertapenem, and vancomycin by the Etest method (AB Biodisk, Solna, Sweden). Erythromycin- or clindamycin-resistant isolates were screened by PCR for the presence of *ermB* and *mefA* resistance genes as previously described (3, 5, 25, 30). Primers 5′-AACCCGAAA AATACGCAAAA-3′ and 5′-ACCCGTTGACTCATTTCCA C-3′ were used to detect the *ermTR* gene. Amplification was performed in a DNA Biometra T-Gradient thermal cycler with one cycle of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 56°C for 90 s, and extension at 72°C for 1 min and a final extension step of 72°C for 10 min. Amplification of DNA from positive controls yielded PCR products of the expected sizes (348, 206, and 152 bp for *mefA*, *ermB*, and *ermTR*, respectively).

A total of 296 invasive neonatal GBS strains were collected during the 2-year period. All strains were highly susceptible to penicillin, ampicillin, and cefotaxime. Likewise, isolates were uniformly susceptible to carbapenems, vancomycin, linezolid, and quinupristin-dalfopristin. Although all of the strains were resistant to gentamicin, none showed high-level resistance (defined as a MIC of >256 μg/ml) (Table 1). Thirty-two isolates were resistant to erythromycin; 17 of these were also resistant to clindamycin. The distribution of macrolide-lincosamide resistance genes according to erythromycin-clindamycin resistance phenotypes is reported in Table 2. Consistent with other reports, methylase was the most common mechanism detected among macrolide-resistant isolates (6, 7, 8, 12). Except for two isolates, all of the isolates with the macrolide-lincosamide-streptogramin B resistance phenotype carried at least one of the *erm* genes. Combinations of resistance genes occurred in 37.5% (12 of 32). One isolate carried all three resistance genes (*ermB*, *ermTR*, and *mefA*).

Since beta-lactam antibiotics remain the first-line treatment for GBS infections (28), a major issue of surveillance is to confirm that invasive isolates in a given country are still susceptible to this class of antibiotics. Intermediate susceptibility to penicillin and ampicillin is rare (1). In the present study, which is the first to report of national data on the antimicrobial susceptibility of GBS in Germany, all of the isolates tested were highly susceptible to penicillin, ampicillin, and cefotaxime. No trend toward reduced penicillin or ampicillin susceptibility of neonatal invasive GBS isolates was observed.

To our knowledge, this study is the first to systematically test for high-level gentamicin resistance in GBS on a larger scale. High-level resistance indicates that the isolate will not be affected synergistically by the combination of penicillin and an aminoglycoside. To date, this type of resistance has been documented in only one GBS strain isolated in France in 1987 (4). None of the strains tested in this study showed high-level resistance to gentamicin.

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TABLE 1. Susceptibility of 296 invasive GBS isolates to different antibiotics

Antibiotic	MIC (μg/ml)			Breakpoints[a] (μg/ml)	% (no./total) Resistant
	Range	50% of isolates	90% of isolates		
Penicillin G	0.016–0.125	0.047	0.064	<0.12	0
Ampicillin	0.016–0.125	0.094	0.094	<0.25	0
Cefotaxime	0.023–0.19	0.047	0.064	<0.5	0
Erythromycin	0.023–>256	0.094	0.94	0.25	10.1 (30/296)
Clindamycin	0.038–>256	0.19	0.25	<0.25	5.7 (17/296)
Gentamicin	32–256	128	128	<1.0	100
Linezolid	0.25–1.5	1.0	1.5	>4[c]	0
Q-D[b]	0.19–1.5	0.50	0.75	<1.0	0
Imipenem	0.004–0.19	0.032	0.064	<0.5	0
Meropenem	0.012–0.094	0.032	0.047	<0.5	0
Ertapenem	0.023–0.094	0.047	0.064	<1.0	0
Vancomycin	0.38–1.0	1.0	1.0	<1.0	0

[a] If not indicated otherwise, NCCLS (19) breakpoints were used. For linezolid and gentamicin, European Committee on Antimicrobial Susceptibility Testing (10) and Deutsche Industrie Norm (9) breakpoints were used, respectively.
[b] Q-D, quinupristin-dalfopristin.
[c] Resistant.

Some newer antibiotics were also included in this study. Linezolid is an oxazolidinone with good activity against gram-positive organisms (14, 21). Quinupristin-dalfopristin is a recently approved streptogramin for the treatment of infections caused by resistant gram-positive bacteria. Ertapenem is a novel carbapenem reported to have activity against gram-positive bacteria similar to that of meropenem (16, 17). Linezolid, ertapenem, and quinupristin-dalfopristin all proved to be highly active in vitro against all of the GBS isolates tested. The MICs obtained were comparable to those reported by others (20, 31). Consequently, all of these agents appear to be promising alternatives for the treatment of GBS infections in selected cases.

Resistance to erythromycin and clindamycin emerged during the last decade in many countries. Macrolides are the recommended second-line agents for GBS prophylaxis in the case of beta-lactam allergy. In this study, 10.1 and 5.7% of the isolates tested were resistant to erythromycin and clindamycin, respectively. Compared to reports from other countries with erythromycin resistance rates of up to 36% and clindamycin resistance rates of up to 18%, our data show lower resistance rates (1, 8, 23). This is of interest and compares well to data on comparably low macrolide resistance of, e.g., pneumococci or group A streptococci in Germany (24). The present study also confirms that *ermB* is the predominant resistance gene in macrolide-resistant GBS isolates with the macrolide-lincosamide-streptogramin B resistance phenotype, whereas the *mef* gene is more common among erythromycin-resistant isolates with the M phenotype. The latter genotype, however, was found at much lower frequencies compared to those reported, e.g., for group A streptococci (13). Of the macrolide-resistant strains, 37.5% harbored combinations of resistance genes. Combinations of resistance genes have been described before only for a small percentage of isolates (6, 8). Only one study reported resistance gene combinations in 29.2% of the isolates (2). To our knowledge, a triple combination of *ermB*, *ermTR*, and *mefA*, which was found in one of our isolates, has not been described previously.

TABLE 2. Distribution of resistance genes among 32 erythromycin- and/or clindamycin-resistant GBS isolates

Resistance[a] (no. of isolates)	No. of isolates	*mefA*	*ermB*	*ermTR*	MIC (μg/ml) (no. of isolates)	
					EM	CM
EM (15)	2	+	−	−	12 μg/ml	
	5	+	−	+	2 (3), 3 (2)	
	6	−	−	+	4 (3), 6 (1), 16 (1), 4 (3), 6 (1), 16 (1), >256 (1)	
	1	+	+	−	3	
	1	+	+	+	4	
EM-CM (15)	9	−	+	−	>256	>256
	4	−	+	+	>256	>256
	1	+	+	−	>256	>256
	1	−	−	−	>256	>256
CM (2)	1	−	+	−		4
	1	−	−	−		2

[a] EM, erythromycin; CM, clindamycin.

In conclusion, our findings demonstrate that GBS isolated from infants in Germany with invasive infections remain uniformly susceptible to penicillin and ampicillin. Routine susceptibility testing for beta-lactams and high-level aminoglycoside resistance of GBS strains isolated in Germany appears unnecessary. The frequency of erythromycin resistance, however, has reached significant levels, while still being remarkably lower than in other parts of the world. With increasing macrolide resistance rates, prophylaxis failure is becoming more likely. Therefore, testing of susceptibility to these drugs should be performed in individual cases when considered as alternatives for prophylaxis and treatment of GBS infection or colonization. Antibiotics such as vancomycin, linezolid, quinupristin-dalfopristin, or carbapenems showed good in vitro activity against GBS, but their use should be strictly limited to cases with an obvious lack of alternatives.

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